# CASC15 mediated upregulation of SERPINE1 by sponging miR-30c- 5p correlates with poor prognosis and immune infiltration of gastric cancer

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# Abstract

**Background:** As a major regulator of plasminogen activator system (PAs), SERPINE1(PAI-1) is associated with poor prognosis in a variety of cancers, but in Stomach adenocarcinoma (STAD), its mechanism of action is not so clear.

**Methods:** Based on RNA Sequencing dataset and survival data for TCGA-STAD, this study screened out genes of significant difference between the gastric cancer group and the normal group using the R language limma package, and carried out a pan-cancer analysis on their expression in combination with the Genotype-Tissue Expression (GTEx) database at the same time. Subsequently, the miRNA and lncRNA associated with high expression level of PAI-1 significantly were identified through the Starbase database in combination with a series of analyses on expression level, correlation and survival. The correlation of immune cell infiltration, biomarkers of immune cells and immune checkpoints between PAI-1 were investigated via TIMER and GEPIA database.

**Results:** PAI-1 was up-regulated significantly in gastric cancer, and was a risk factor and could indicate the survival of STAD patients. According to analysis results, CASC15/hsa-miR-30c-5p/ PAI-1 axis may be the most potential regulatory network of PAI-1 related to survival in gastric cancer. And the expression level of PAI-1 in gastric cancer had a positive correlation with immune cell infiltration, biomarkers of immune cells and immune checkpoints obviously.

**Conclusion:** Collectively, PAI-1 could accurately indicate prognosis, evaluate the tumor immune infiltration, probability participate in the regulation of gastric cancer through CASC15/miR-30c-5p/ PAI-1 axis, and PAI-1 might exert its oncogenic roles through increasing tumor immune cell infiltration and immune checkpoint expression.


# Introduction

Globally, as the fifth most common malignancy, gastric cancer is also the third most important cancer-causing death. The high-risk factors for gastric cancer include helicobacter pylori infection, poor diet, age and high salt intake.(1)The combination of surgery, chemotherapy and targeted therapy has prolonged the overall survival of patients with gastric cancer significantly, and improved their quality of life as well. However, most of the patients with gastric cancer have been at an advanced stage with poor prognosis once confirmed as the disease for lack of obvious or specific symptoms in an early stage. (2) Therefore, it is an urgent to find new therapeutic targets or prognostic biomarkers.

As a serine protease inhibitor, SERPINE1(also known as plasminogen activator inhibitor type 1[PAI-1]), is a member of the urokinase plasminogen activator (uPA) system(3)that regulates extracellular fibrinolysis and influences cell invasion and migration, as well as ECM remodeling.(4)PAI-1 is closely related to the occurrence, development, invasion and metastasis of various cancers. PAI-1 induces migration and invasion of esophageal squamous cell carcinoma (ESCC) cells and macrophages by low-density lipoprotein receptor-related protein 1 (LRP1) of PAI-1 receptor based on Akt and ERK1/2 signaling

pathways, thus promoting the progression of ESCC.(5) On the one hand, it is associated with poor prognosis in patients with different types of tumors, including breast cancer.(6) On the other hand, the expression of PAI-1 may promote angiogenesis and migration and anti-apoptosis, thus regulating the proliferation of cancer cells and support the tumorigenesis.(7) Similarly, some study has also identified PAI-1 in pancreatic tumor, which is closely related to venous thromboembolism for pancreatic cancer.(8) A study has also suggested that in an oxygen-deficient environment, PAI-1 can induce a malignant phenotype of tumor as hypoxia-inducible factor through activating or enhancing JAK-STAT signaling pathway, TGF-β signaling pathway and NOTCH signaling pathway.(9)However, the prognosis, expression and relevant mechanism of PAI-1 in gastric cancer, as well as its relation with immune infiltration of gastric cancer, are still to be further investigated.

In this study, the TCGA-STAD dataset and GTEx dataset were utilized to analyze the expression, prognosis and survival of PAI-1 in gastric cancer, as well as its expression in various cancers. At the same time, the Starbase database was used to analyze PAI-1 related lncRNA and miRNA based on the sponge regulation mechanism of ceRNA, and thus, the lncRNA/miRNA/mRNA axis was determined. In addition, the study found that PAI-1 was positively correlated with immune cell infiltration, immune checkpoints, and biomarkers of immune cells in gastric cancer. The results suggested that PAI-1 regulated by lncRNA through ceRNA mediation may be a new biomarker for the treatment of gastric cancer.

# Methods

### Data download, processing and differential analysis

The genomic and clinicopathological information of 33 cancers were obtained from The Cancer Genome Atlas (TCGA) database (https://genome-cancer.ucsc.edu/), and 18 tumors (BLCA、BRCA、CHOL、COAD、ESCA、GBM、HNSC、KICH、KIRC、KIRP、LIHC、LUAD、LUSC、PRAD、READ、STAD、THCA and UCEC) types with normal samples larger than 5 were further analyzed. A differential analysis of PAI-1 in the above cancers was performed using the R Package limma.(10) If $P < 0.05$, there was statistical significance. At the same time, differentially expressed genes（DEGs）in gastric cancer tissues and normal tissues were screened out by using R Package limma. The critical condition for screening was $|log2FC| \geq 1.0$ and adj. $P < 0.05$, with p value corrected by the false discovery rate (FDR) method. Then, the survival package in R was used for a prognostic survival analysis on significant DEGs in gastric cancer. p-value < 0.05 were considered significant.

### Analysis on GEPIA database

As a database, GEPIA (http://gepia.cancer-pku.cn/) is a web-based tool for cancer or normal gene-expression profiling and interaction analysis based on TCGA and genotype-tissue expression (GTEx) data.(11)The expression of PAI-1 in 18 different cancers and normal tissues was investigated using GEPIA database. p-value < 0.05 were considered significant. Furthermore, 11 cancers types were analyzed for survival outcomes (including OS and RFS). In addition, GEPIA was also used for a survival analysis on upstream lncRNAs of PAI-1. Log rank p value <0.05 was considered as statistically significant. Finally,

GEPIA was also used for evaluating the correlation of PAI-1 with the biomarkers of immune cells and immune checkpoints (CD274, CTLA4 and PDCD1). $P < 0.05$ and $|R| > 0.1$ as the thresholds, and if relevant conditions were met, there was statistical significance.

### Prediction of candidate miRNA by Starbase database

Upstream miRNAs that may bind to PAI-1 were predicted by 7 prediction programs of target genes embedded in Starbase 2.0(https://rna.sysu.edu.cn/) online site(12), i.e., PitA, RNA22, miRmap, microT, Miranda, PicTar and TargetScan. Only miRNAs obtained by 3 or more target gene prediction programs can be used as candidate miRNAs.

### Screening candidate miRNA and constructing miRNA - PAI-1 correlation analysis

The expression data of miRNA in gastric cancer were downloaded from TCGA database. The R language limma package was used to screen out candidate miRNAs different in expression in gastric cancer tissues and normal tissues. p-value < 0.05 were considered statistically significant. Then, the spearman correlation coefficient method(13) was used to analyze the correlation between candidate miRNA and PAI-1, with $P < 0.05$ and $|R| > 0.1$ as the thresholds for screening. R language ggpubr package and ggExtra package were used to map the correlation between the candidate miRNA and PAI-1 expression level. Finally, Cytoscape software (V.3.8.0) was used to visualize the miRNA-PAI-1 regulatory network conforming to the above screening conditions.(14)

### Kaplan and Meier plotter analysis

Kaplan and Meier plotter database (http://kmplot.com/analysis/) may be used to assess the effects of genes or miRNAs on survival in more than 20 cancers, including liver cancer.(15) The database was used for a survival analysis on hsa-miR-30c-5p in gastric cancer. For logrank. Log rank p-value < 0.05 were considered statistically significant.

### LncRNA prediction and correlation analysis

The upstream lncRNAs of miR-30c-5p were predicted by miRNA-lncRNA module group of Starbase database. Screened lncRNAs were visualized by using Cytoscape software. Differentially expressed lncRNAs between gastric cancer tissues and normal tissues were obtained by a difference analysis of candidate lncRNAs using R language Limma package. p-value < 0.05 were considered statistically significant. Then, the spearman correlation coefficient method was used for a correlation analysis on differentiated lncRNAs and miR-30c-5p. $P < 0.05$ and $|R| > 0.1$ were considered statistically significant. Finally, the lncRNAs that had been screened out were used for a correlation analysis with PAI-1. In addition, the expression boxplots of identified lncRNAs in gastric cancer tissues and normal tissues were charted out by utilizing ggboxplot package.

### LncRNA survival analysis and lncRNA-miRNA-mRNA network construction

In combination with clinical data of TCGA-STAD, a survival analysis on lncRNAs that had been screened out was performed using R language survival and survminer packages. At last, the GEPIA database was used for another survival analysis on the obtained lncRNAs, so as to further screen prognostic lncRNAs.

### Immune cell infiltration analysis

TIMER (https://cistrome.shinyapps.io/timer/) Database is a web server for comprehensive analysis of tumor-infiltrating immune cells.(16) TIMER software was used to analyze the correlation of PAI-1 expression level in gastric cancer with immune cell infiltration level and immune checkpoint expression level. p-value < 0.05 were considered statistically significant. Then, the spearman correlation coefficient method was used to analyze the correlation between PAI-1 and biomarkers of immune cells. To evaluate the difference in infiltration of 22 types of immune cells between low expression group and high expression group of PAI-1, CiberSort (https://cibersort.stanford.edu/about.php) was used for estimate immune cells proportion in tumor tissues accurately.(17)p-value < 0.05 were considered statistically significant. At the same time, the spearman correlation coefficient method was used to analyze the correlation between PAI-1 expression level and the infiltration level of 22 types of immune cells. If $P < 0.05$ and $|R| > 0.1$, there was correlation. Venn diagram was then used for acquiring the intersection, and thus, immune cells highly correlated with PAI-1 expression level were obtained.

# Results

### An analysis on the expression of PAI-1 in pan-cancer

To evaluate the potential function and expression of PAI-1 in a variety of cancers, the study analyzed the expression of PAI-1 first, as shown in **Fig.1**. The expression level of PAI-1 in BRCA, COAD, ESCA, GBM, HNSC, KIRC, READ, STAD or THCA was significantly higher than in normal tissue. However, the level was significantly down-regulated in CHOL, KICH, KIRP, or LIHC. There was no significant difference for the level in BLCA, LUAD, LUSC, PRAD and UCEC. Similarly, the mRNA expression data of gastric cancer were analyzed, and through a combination of Kaplan-Meier analysis and COX regression model, 82 survival-related genes highly correlated with prognosis of gastric cancer were screened out (**Supplementary Table S1**), including high-risk PAI-1. Subsequently, the expression of PAI-1 in various cancer tissues was verified by the GEPIA database. As shown in **Fig.2A-2H**, the expression was up-regulated obviously in BRCA, COAD, ESCA, GBM, HNSC, KIRC, READ, and STAD, while down-regulated in KICH, KIRP, LIHC, LUSC, THCA, and UCEA (**Fig.2I-2N**). Overall, the expression levels of PAI-1 in BRCA, COAD, ESCA, GBM, HNSC, KIRC, READ, and STAD obviously increased, while in KICH, KIRP, and LIHC decreased, indicating that PAI-1 might be closely related to the occurrence, development, invasion and metastasis of these 11 cancers.

### An analysis on the prognosis of PAI-1 in various cancers

The survival analysis for PAI-1 in these 11 cancers was conducted based on the GEPIA online database. As shown in **Fig.3** and **Fig.4**, in terms of overall survival (OS), the high expression levels of PAI-1 in HNSC, STAD, KIRP and LIHC indicated a poor prognosis, while in terms of recurrence-free survival (RFS), the up-

regulation of PAI-1 expression in GBM, KIRC, STAD, KICH and KIRP represented a poor prognosis. In combination with the expression levels of PAI-1 in cancer tissues and normal tissues, the study found that only the up-regulation of PAI-1 expression level worsened the prognosis of gastric cancer, and there was no statistical significance for the survival prediction related to other malignant tumors. To sum up, as a potential prognostic biomarker, PAI-1 may indicate poor prognosis of gastric cancer.

### Prediction and analysis on upstream miRNAs of PAI-1

The upstream miRNAs of PAI-1 were predicted based on the Starbase online database, and the miRNAs that could be predicted by three databases simultaneously were captured for further analysis. According to the mechanism of action of miRNA on target gene mRNA, miRNA should be negatively correlated with target gene. Subsequently, the correlation between PAI-1 and these miRNAs was calculated, and at last, 10 closely related miRNAs were obtained. As shown in **Fig.5A**, a miRNA- PAI-1 regulatory network that met the above conditions for screening was visualized through Cytoscape software, and the data concerning correlation between the 10 miRNAs and PAI-1 were described in **Table 1**. According to the theory, candidate miRNAs should be poorly expressed in gastric cancer tissues compared to normal tissues, while only miR-30e-5p and miR-30c-5p conformed. Meanwhile, the expression levels of miR-30e-5p and miR-30c-5p in gastric cancer tissues and normal tissues were detected, as shown in **Fig. 5B-5C**. Among the candidate miRNAs, only miR-30c-5p had expression level lowered in gastric cancer with statistical significance. As shown in **Fig.5D**, Also, a survival analysis on miR-30c-5p in gastric cancer based on the Kaplan-Meier plotter database found that up-regulation of miR-30c-5p expression level in gastric cancer was associated with poor prognosis of gastric cancer. All these findings suggested that miR-30c-5p might be the most promising upstream regulatory gene in gastric cancer.

Table 1. The expression correlation between predicted miRNAs and SERPINE1 in STAD

| Gene | miRNA | R value | pvalue | logFC |
|---|---|---|---|---|
| SERPINE1 | miR-19b-3p | -0.223967703 | 1.36E-05 | 1.285571282 |
| SERPINE1 | miR-19a-3p | -0.206224287 | 6.37E-05 | 1.901414418 |
| SERPINE1 | miR-30d-5p | -0.200104292 | 0.000105202 | 0.273812907 |
| SERPINE1 | miR-30b-5p | -0.172364077 | 0.000856495 | 0.261855105 |
| SERPINE1 | miR-148a-3p | -0.171064971 | 0.000938155 | 0.448863061 |
| SERPINE1 | miR-30c-5p | -0.1603171 | 0.001945506 | -0.162681261 |
| SERPINE1 | miR-196b-5p | -0.156509062 | 0.002493569 | 4.694990524 |
| SERPINE1 | miR-30e-5p | -0.14994663 | 0.003777251 | -0.030222247 |
| SERPINE1 | miR-942-5p | -0.115381271 | 0.026106396 | 1.051408458 |
| SERPINE1 | miR-196a-5p | -0.105013598 | 0.042986363 | 4.890843882 |

### Prediction and analysis on the upstream lncRNAs of miR-30c-5p

According to the competitive endogenous RNA hypothesis, lncRNA may be involved in the expression and regulation of target genes through competitive binding with miRNA. Through miRNA, the lncRNA that can bind to it may be derived reversely based on this mechanism. Thus, a total of 101 lncRNAs that may bind to miR-30c-5p were obtained through a prediction based on the Starbase database, and the lncRNA-miR-30c-5p regulatory network was constructed using Cytoscape software (**Supplementary Fig.1**). A screening based on the mechanism of competitive binding with miRNA identified 6 lncRNAs(GATA3-AS1, NORAD, KCNH7-AS1, LINC02863, CASC15, and LINC01094) expression up-regulated significantly in gastric cancer as compared with those in the normal group and significantly correlated with miR-30c-5p ($P < 0.05$, $|R| > 0.1$). For their expression levels, shown **Fig.6A-6F**. Subsequently, the 6 lncRNAs were performed correlation analysis with PAI-1, and the results were as shown in **Table 2**. Obviously, the p value for correlation of GATA3-AS1, NORAD, and KCNH7-AS1 with PAI-1 was greater than 0.05, NORAD was negatively correlated with PAI-1, and therefore, there was no statistical significance. Next, LINC02863, CASC15 and LINC01094 were analyzed for survival, and the results showed that in gastric cancer with high expression levels of CASC15 and LINC01094, the OS was poor. Finally, again with the GEPIA database, the prognosis of CASC15 and LINC01094 in gastric cancer was verified. As shown in **Figure 7**, only patients with high expression level of CASC15 in gastric cancer had poor OS and RFS, indicating that the up-regulation of CASC15 expression level was related to the poor prognosis of gastric cancer. Therefore, CASC15 may be the most promising upstream lncRNA of miR-30c-5p/ PAI-1.

**Table 2.** Correlation analysis between lncRNA and miR-30c-5p or lncRNA

and SERPINE1 in STAD

| lncRNA | miRNA | R value | p value |
|---|---|---|---|
| GATA3-AS1 | miR-30c-5p | -0.115965869[a] | 0.02530644* |
| NORAD | miR-30c-5p | -0.166618219[a] | 0.001275214* |
| LINC02863 | miR-30c-5p | -0.129356938[a] | 0.012523149* |
| CASC15 | miR-30c-5p | -0.127152482[a] | 0.014169844* |
| LINC01094 | miR-30c-5p | -0.146472635[a] | 0.004676307* |
| KCNH7-AS1 | miR-30c-5p | -0.116009984[a] | 0.025250466* |
| lncRNA | Gene | R value | p value |
| GATA3-AS1 | SERPINE1 | 0.096288048 | 0.063567773 |
| NORAD | SERPINE1 | -0.014045404 | 0.787063572 |
| LINC02863 | SERPINE1 | 0.109654194[a] | 0.034498615* |
| CASC15 | SERPINE1 | 0.294656742[a] | 8.16E-09* |
| LINC01094 | SERPINE1 | 0.321744257[a] | 2.65E-10* |
| KCNH7-AS1 | SERPINE1 | 0.001144345 | 0.982450299 |

[a]These results are statistically significant

*p value < 0.05

## PAI-1 positively correlates with immune cell infiltration in gastric cancer

The copy number variation (CNV) of gene, a variant form of DNA mutation, has been reported to be closely related to human tumors.The study analyzed the relations of various copy numbers of PAI-1 with B cell, CD8+T cell, CD4+T cell, macrophage, neutrophil, or dendritic cell infiltration degree using TIMER database. The CNV degree was indicated by Deep Deletion, Arm−level Deletion, Diploid/Normal, Arm−level Gain and high Amplification. As shown in **Fig.8**, in gastric cancer with CNV of PAI-1, except for deep deletion, the infiltration degree of most immune cells was significantly reduced, indicating that the PAI-1 might mediated immune cells infiltration. Also, the correlation between PAI-1 and the infiltration degree of the 6 types of immune cells was verified through tumor purity correction. As shown in **Fig.9B and Fig.9D-9F**, the expression level of PAI-1 had a significant positive correlation with the infiltration degree of CD8+T cell, Macrophage, Neutrophil, and Dendritic cell. As shown in **Fig.9A and Fig.9C**, the correlation between B cell, CD4+T cell and expression level of PAI-1 was no statistical significance. Therefore, CiberSort was further used to analyze the proportion of each of the 22 types of immune cells in all samples (**Fig.10**). Then, PAI-1 was divided into high and low expression groups according to the corresponding expression levels, and the violin diagram was used to observe the difference in infiltration

degree of various immune cells for different PAI-1 expression levels. The results showed that the infiltration degrees of NK cells resting, Monocytes, Dendritic cells activated, Mast cells activated, Eosinophils, and Neutrophils in the high PAI-1 expression group were significantly increased as compared with those in the low PAI-1 expression group, and the infiltration degree of NK cells activated in the high PAI-1 expression group was significantly decreased as compared with that in the low PAI-1 expression group (**Fig.11A**). Furthermore, as shown in **Fig.11B**, in the analysis concerning correlation between PAI-1 expression level and immune cell infiltration degree, the PAI-1 expression level had a significant positive correlation with the infiltration degrees of NK cells resting, Monocytes, Dendritic cells activated, Mast cells activated, Eosinophils, and Neutrophils, and a negative correlation with the infiltration degree of NK cells activated. Two methods indicated that the infiltration degrees of these seven immune cells were correlated with PAI-1.

### Correlation between PAI-1 expression level and biomarkers of immune cells in gastric cancer

To further explore the immunization of PAI-1 in gastric cancer, the Spearman's rank correlation coefficient method was used for analyzing the correlation between PAI-1 and biomarkers of immune cells. As shown in **Table 3**, PAI-1 had a significant positive correlation with CD8+T cell's biomarker (CD8A), CD4+T cell's biomarker (CD4), M1 macrophage's biomarkers (IRF5 and PTGS2), M2 macrophage's biomarkers (CD163, VSIG4 and MS4A4A), neutrophil's biomarkers (ITGAM, CCR7) and dendritic cell's biomarkers (HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1, NRP1, and ITGAX). Based on the table, the correlation between the PAI-1 expression in gastric cancer tissues and the biomarkers of immune cells was also verified through GEPIA database, and the results showed that PAI-1 had a significant positive correlation with B cell's biomarker (CD79A), CD8+T cell's biomarkers (CD8A, CD8B), CD4+T cell's biomarker (CD4), M1 macrophage's biomarkers (IRF5 and PTGS2), M2 macrophage's biomarkers (CD163, VSIG4 and MS4A4) A), neutrophil's biomarkers (ITGAM, CCR7) and dendritic cell's biomarkers (HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1, CD1C, NRP1, and ITGAX). Generally, these conclusions have supported the positive correlation between PAI-1 expression and immune cells infiltration.

**Table 3.** Correlation analysis between SERPINE1 and biomarkers of immune cells in STAD determined by Spearman's correlation analysis and GEPIA database.

| ImmuneCell | Biomarker | Spearman's correlation | | GEPIA | |
|---|---|---|---|---|---|
| | | P value | R value | P value | R value |
| B cell | CD19 | 0.63845434 | 0.024330413 | 0.059 | 0.094 |
| | CD79A | 0.060862322 | 0.096902492 | 0.029 | 0.11 |
| CD8+ T cell | CD8A | 0.00217794 | 0.157962453 | 0.0003 | 0.18 |
| | CD8B | 0.241480665 | 0.060616452 | 0.034 | 0.11 |
| CD4+ T cell | CD4 | 2.15E-06 | 0.242599158 | 3.70E-09 | 0.29 |
| M1 macrophage | NOS2 | 0.779449455 | -0.01450199 | 0.72 | -0.018 |
| | IRF5 | 0.036128571 | 0.108277847 | 0.00013 | 0.19 |
| | PTGS2 | 3.83E-13 | 0.365259757 | 3.80E-19 | 0.42 |
| M2 macrophage | CD163 | 2.01E-13 | 0.369159176 | 6.80E-16 | 0.39 |
| | VSIG4 | 5.28E-12 | 0.348344294 | 4.40E-17 | 0.4 |
| | MS4A4A | 4.27E-09 | 0.298813289 | 1.20E-12 | 0.34 |
| Neutrophil | CEACAM8 | 0.781027213 | 0.014402196 | 0.21 | 0.063 |
| | ITGAM | 7.07E-09 | 0.29469496 | 1.10E-11 | 0.33 |
| | CCR7 | 8.83E-06 | 0.227836842 | 5.50E-08 | 0.26 |
| Dendritic cell | HLA-DPB1 | 0.027667102 | 0.113754238 | 0.002 | 0.15 |
| | HLA-DQB1 | 0.006184357 | 0.141246331 | 0.015 | 0.12 |
| | HLA-DRA | 0.03018002 | 0.111993173 | 0.01 | 0.13 |
| | HLA-DPA1 | 0.026490874 | 0.114626237 | 0.0075 | 0.13 |
| | CD1C | 0.073171952 | 0.092639663 | 0.013 | 0.12 |
| | NRP1 | 0 | 0.486063716 | 6.40E-31 | 0.53 |
| | ITGAX | 6.62E-12 | 0.346809648 | 7.30E-16 | 0.38 |

### Association of PAI-1 with common immune checkpoints in gastric cancer tissues

The commonly used immune checkpoints, including PD1, CD274 and CTLA4, are important checkpoints in the tumor immune escape mechanism. Based on the above analyses, it can be preliminarily assumed that PAI-1 has a potential for guiding the occurrence and development of gastric cancer. Therefore, the correlation of PAI-1 with PD1, CD274 and CTLA4 was analyzed using TIMER after tumor purity correction. The results showed that PAI-1 had a significant positive correlation with PD1, CD274 and CTLA4 (**Fig.12A-12C**). The validation of GEPIA database has also confirmed the above results (Figure 12D-12F). The

results have supported the involvement of tumor immune escape mechanism in gastric cancer development regulated by PAI-1.

# Discussion

By now, the morbidity and mortality of gastric cancer have always been at a high level with poor prognosis. For this, it is urgent to find new solutions. Therefore, the study on the molecular mechanism of gastric cancer is conducive to the search for potential biomarkers and the development of new therapeutic targets. At present, with the development of bioinformatics, the bioinformatics analysis method has been widely used to screen and analyze the genes related to the progression and prognosis of various cancers, and has provided new ideas for improving the studies on tumor molecular mechanism and exploring new targeted therapies.

More and more studies have shown that PAI-1 plays an important role in many cancers.(7, 18-20) Studies have shown that the high expression of PAI-1 in gastric cancer may promote the occurrence and development of gastric cancer, and at the same time, it is associated with the poor prognosis of gastric cancer.(21, 22)However, the specific mechanism of PAI-1 in the occurrence and development of gastric cancer is still to be further clarified. Therefore, this study carried out a pan-cancer analysis of PAI-1 using TCGA database firstly, and identified highly expressed PAI-1 in a variety of cancers. Based on the GEPIA database, a survival analysis was conducted for the cancers with high PAI-1 expression levels, and the results showed that the high PAI-1 expression was correlated with the poor prognosis of patients with tumors obviously, especially in those with gastric cancer, the OS and RFS were significantly decreased among the patients with high PAI-1 expression.

According to relevant reports, non-coding genes including lncRNAs, miRNAs and circRNAs, may be involved in the in the regulation of mRNA through the ceRNA regulation mechanism, thus playing an important role in a variety of cancers.(23-25)The microRNAs are a category of small non-coding RNAs of approximate length 22 nucleotides that degrade mRNAs or inhibit mRNAs expression mainly through binding to miRNA response element (MRE) on target RNA transcript. Under some conditions, miRNA may occasionally enhance gene expression or increase the target gene expression level.(26) In order to identify miRNAs that may bind to PAI-1, 7 different versions of miRNA software were used for relevant prediction, and after screening based on correlation, 10 miRNAs were obtained at last. Theoretically, due to the up-regulation of PAI-1 expression level, the expression levels of miRNAs bound to PAI-1 will be down-regulated. Therefore, a further survival analysis was conducted using miR-30c-5p. The results showed that the increased expression level of miR-30c-5p in gastric cancer was correlated with the poor prognosis of gastric cancer significantly. Studies have shown that miR-30c-5p can inhibit cell growth and proliferation through targeting RAB32, and reducing the expression level of miR-30c-5p may promote the development of liver cancer.(27) Some studies have shown that the expression level of miR-30c-5p in gastric cancer is significantly reduced, and its downregulation may advance the capacities of gastric cancer cells for migration and invasion. Further studies have shown that through inhibiting the expression of its target transfer-related protein 1 (MTA1), miR-30c-5p can inhibit epithelial-to-

mesenchymal transformation (EMT), an important process in gastric cancer metastasis.(28) Therefore, miR-30c-5p was selected as one of the most promising upstream targets of PAI-1.

Based on the hypothesis of ceRNA regulation, lncRNA can be used as endogenous RNA to adsorb miRNA, thus participating in the regulation of cancer tissues.(29) Therefore, at the upstream of miR-30c-5p/PAI-1 axis there should be carcinogenic lncRNA. Six significantly correlated lncRNAs that may bind to miR-30c-5p were identified through a prediction based on Starbase database. Then, through correlation analysis between lncRNAs and PAI-1, 3 candidate lncRNAs were screened out for survival analysis. At last, the most promising upstream lncRNA-CASC15 was obtained through a verification based on GEPIA database. Several studies have shown that CASC15 is highly expressed in liver cancer, lung cancer, tongue squamous carcinoma, gastric cancer, colorectal cancer, cervical cancer and breast cancer with carcinogenicity.(30)According to relevant reports, in gastric cancer, CASC15 is involved in the occurrence of gastric cancer when it interacts with EZH2 and WDR5 to regulate CDKN1A in the nucleus. Based on this molecular axis, cell apoptosis may be induced to promote or inhibit cell proliferation through up-regulation or down-regulation of CASC15, and cell migration and invasion may be enhanced or inhibited through influencing the EMT process.(31) In summary, CASC15/ miR-30c-5p/ PAI-1 axis may be a potential regulatory network in gastric cancer.

Numerous studies have proved that tumor immune cell infiltration may influence the effect of chemotherapy, radiotherapy or immunotherapy and the prognosis of patients with tumors.(32, 33) Based on the TIMER database, the study has found that PAI-1 has a significant positive correlation with CD8+T cells, Macrophages, Neutrophils and Dendritic cells in gastric cancer. Moreover, PAI-1 is also positively correlated with most of the biomarkers of immune cells. These results have suggested that PAI-1 expression is closely related to immune cell infiltration in tumors. This may partially explain the carcinogenesis of the PAI-1.

Immunotherapy is not only dependent on the proportion of immune cell infiltration in tumor microenvironment, but also associated with the expression of tumor immune checkpoint.(34-36) Immune checkpoint is important for tumor immune escape mechanism.(37, 38)Therefore, the relationship between immune checkpoint and PAI-1 in gastric cancer was also analyzed. The results showed that the high expression of PAI-1 had a significant positive correlation with PD1, CD274 and CTLA4. Therefore, targeting PAI-1 may improve the effect of immunotherapy for gastric cancer indirectly.

In our study, the high expression of PAI-1 in a variety of human cancers was verified, and several analyses showed that the high expression of PAI-1 was correlated with poor prognosis of gastric cancer significantly. Also, the upstream miRNA and lncRNA of PAI-1 were predicted, and CASC15/ miR-30c-5p/ PAI-1 regulatory network was constructed. In addition, the relationship between PAI-1 and immune cell infiltration in gastric cancer was explored, and the results suggested that PAI-1 mediated the occurrence or progression of gastric cancer by changing tumor microenvironment and enhancing tumor immune escape mechanism.

# Abbreviations

PAI-1

STAD Stomach adenocarcinoma

GTEx Genotype-Tissue Expression

TCGA The Cancer Genome Atlas

TCGA-STAD The Cancer Genome Atlas-Stomach adenocarcinoma

DEGs Differentially expressed genes

OS overall survival

RFS recurrence-free survival

CNV copy number variation

# Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

TCGA gene expression profiles and patients' clinical data could be acquired from TCGA data portal(https://portal.gdc.cancer.gov/). Part of genome analyses could be obtained from GEPIA (http://gepia2.cancer-pku.cn/), Kaplan and Meier plotter database (http://kmplot.com/analysis/). Prediction on the upstream miRNA and lncRNA could be obtained from Starbase 2.0(https://rna.sysu.edu.cn/).Immune cell infiltration analysis could be obtained CiberSort (https://cibersort.stanford.edu/about.php) and TIMER (https://cistrome.shinyapps.io/timer/).

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript. F Y and J X: conceptualization and methodology. J X and K L: software and data curation. J X and K L: validation. J X: original draft preparation. F Y and J X: review, editing, and supervision.

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## References

1. Smyth EC, Nilsson M, Grabsch HI, van Grieken NCT, Lordick F. Gastric cancer. The Lancet. 2020;396(10251):635-48.
2. Wei L, Sun J, Zhang N, Zheng Y, Wang X, Lv L, et al. Noncoding RNAs in gastric cancer: implications for drug resistance. Mol Cancer. 2020;19(1):62.
3. Smith HW, Marshall CJ. Regulation of cell signalling by uPAR. Nature reviews Molecular cell biology. 2010;11(1):23-36.
4. Duffy MJ. The urokinase plasminogen activator system: role in malignancy. Current pharmaceutical design. 2004;10(1):39-49.
5. Sakamoto H, Koma YI, Higashino N, Kodama T, Tanigawa K, Shimizu M, et al. PAI-1 derived from cancer-associated fibroblasts in esophageal squamous cell carcinoma promotes the invasion of cancer cells and the migration of macrophages. Lab Invest. 2021;101(3):353-68.
6. Li S, Wei X, He J, Tian X, Yuan S, Sun L. Plasminogen activator inhibitor-1 in cancer research. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2018;105:83-94.
7. Kubala MH, DeClerck YA. The plasminogen activator inhibitor-1 paradox in cancer: a mechanistic understanding. Cancer metastasis reviews. 2019;38(3):483-92.
8. Hisada Y, Garratt KB, Maqsood A, Grover SP, Kawano T, Cooley BC, et al. Plasminogen activator inhibitor 1 and venous thrombosis in pancreatic cancer. Blood Adv. 2021;5(2):487-95.
9. Pei JP, Zhang CD, Yusupu M, Zhang C, Dai DQ. Screening and Validation of the Hypoxia-Related Signature of Evaluating Tumor Immune Microenvironment and Predicting Prognosis in Gastric Cancer. Front Immunol. 2021;12:705511.
10. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic acids research. 2015;43(7):e47.

11. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. Nucleic acids research. 2019;47(W1):W556-w60.
12. Li JH, Liu S, Zhou H, Qu LH, Yang JH. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic acids research. 2014;42(Database issue):D92-7.
13. de Winter JC, Gosling SD, Potter J. Comparing the Pearson and Spearman correlation coefficients across distributions and sample sizes: A tutorial using simulations and empirical data. Psychological methods. 2016;21(3):273-90.
14. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome research. 2003;13(11):2498-504.
15. Nagy Á, Lánczky A, Menyhárt O, Győrffy B. Validation of miRNA prognostic power in hepatocellular carcinoma using expression data of independent datasets. Scientific reports. 2018;8(1):9227.
16. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. Cancer research. 2017;77(21):e108-e10.
17. Chen B, Khodadoust MS, Liu CL, Newman AM, Alizadeh AA. Profiling Tumor Infiltrating Immune Cells with CIBERSORT. Methods in molecular biology (Clifton, NJ). 2018;1711:243-59.
18. Saidak Z, Soudet S, Lottin M, Salle V, Sevestre MA, Clatot F, et al. A pan-cancer analysis of the human tumor coagulome and its link to the tumor immune microenvironment. Cancer immunology, immunotherapy : CII. 2021;70(4):923-33.
19. Akula SM, Ruvolo PP, McCubrey JA. TP53/miR-34a-associated signaling targets SERPINE1 expression in human pancreatic cancer. Aging. 2020;12(3):2777-97.
20. McCann JV, Xiao L, Kim DJ, Khan OF, Kowalski PS, Anderson DG, et al. Endothelial miR-30c suppresses tumor growth via inhibition of TGF-β-induced Serpine1. The Journal of clinical investigation. 2019;129(4):1654-70.
21. Teng F, Zhang JX, Chen Y, Shen XD, Su C, Guo YJ, et al. LncRNA NKX2-1-AS1 promotes tumor progression and angiogenesis via upregulation of SERPINE1 expression and activation of the VEGFR-2 signaling pathway in gastric cancer. Molecular oncology. 2021;15(4):1234-55.
22. Sakakibara T, Hibi K, Koike M, Fujiwara M, Kodera Y, Ito K, et al. PAI-1 expression levels in gastric cancers are closely correlated to those in corresponding normal tissues. Hepato-gastroenterology. 2008;55(85):1480-3.
23. Lakshmi S, Hughes TA, Priya S. Exosomes and exosomal RNAs in breast cancer: A status update. European journal of cancer (Oxford, England : 1990). 2021;144:252-68.
24. Wang JY, Yang Y, Ma Y, Wang F, Xue A, Zhu J, et al. Potential regulatory role of lncRNA-miRNA-mRNA axis in osteosarcoma. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2020;121:109627.
25. Volovat SR, Volovat C, Hordila I, Hordila DA, Mirestean CC, Miron OT, et al. MiRNA and LncRNA as Potential Biomarkers in Triple-Negative Breast Cancer: A Review. Frontiers in oncology.

2020;10:526850.
26. Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annual review of biochemistry. 2010;79:351-79.
27. He Z, Tian M, Fu X. Reduced expression of miR-30c-5p promotes hepatocellular carcinoma progression by targeting RAB32. Mol Ther Nucleic Acids. 2021;26:603-12.
28. Cao JM, Li GZ, Han M, Xu HL, Huang KM. MiR-30c-5p suppresses migration, invasion and epithelial to mesenchymal transition of gastric cancer via targeting MTA1. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2017;93:554-60.
29. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell. 2011;146(3):353-8.
30. Gu X, Chu Q, Zheng Q, Wang J, Zhu H. The dual functions of the long noncoding RNA CASC15 in malignancy. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2021;135:111212.
31. Wu Q, Xiang S, Ma J, Hui P, Wang T, Meng W, et al. Long non-coding RNA CASC15 regulates gastric cancer cell proliferation, migration and epithelial mesenchymal transition by targeting CDKN1A and ZEB1. Molecular oncology. 2018;12(6):799-813.
32. Waniczek D, Lorenc Z, Śnietura M, Wesecki M, Kopec A, Muc-Wierzgoń M. Tumor-Associated Macrophages and Regulatory T Cells Infiltration and the Clinical Outcome in Colorectal Cancer. Archivum immunologiae et therapiae experimentalis. 2017;65(5):445-54.
33. Zhang H, Liu H, Shen Z, Lin C, Wang X, Qin J, et al. Tumor-infiltrating Neutrophils is Prognostic and Predictive for Postoperative Adjuvant Chemotherapy Benefit in Patients With Gastric Cancer. Annals of surgery. 2018;267(2):311-8.
34. Chae YK, Arya A, Iams W, Cruz MR, Chandra S, Choi J, et al. Current landscape and future of dual anti-CTLA4 and PD-1/PD-L1 blockade immunotherapy in cancer; lessons learned from clinical trials with melanoma and non-small cell lung cancer (NSCLC). Journal for immunotherapy of cancer. 2018;6(1):39.
35. Xu W, Atkins MB, McDermott DF. Checkpoint inhibitor immunotherapy in kidney cancer. Nature reviews Urology. 2020;17(3):137-50.
36. Atkins MB, Clark JI, Quinn DI. Immune checkpoint inhibitors in advanced renal cell carcinoma: experience to date and future directions. Annals of oncology : official journal of the European Society for Medical Oncology. 2017;28(7):1484-94.
37. Ruiz de Galarreta M, Bresnahan E, Molina-Sánchez P, Lindblad KE, Maier B, Sia D, et al. β-Catenin Activation Promotes Immune Escape and Resistance to Anti-PD-1 Therapy in Hepatocellular Carcinoma. Cancer discovery. 2019;9(8):1124-41.
38. O'Donnell JS, Teng MWL, Smyth MJ. Cancer immunoediting and resistance to T cell-based immunotherapy. Nature reviews Clinical oncology. 2019;16(3):151-67.

# Figures

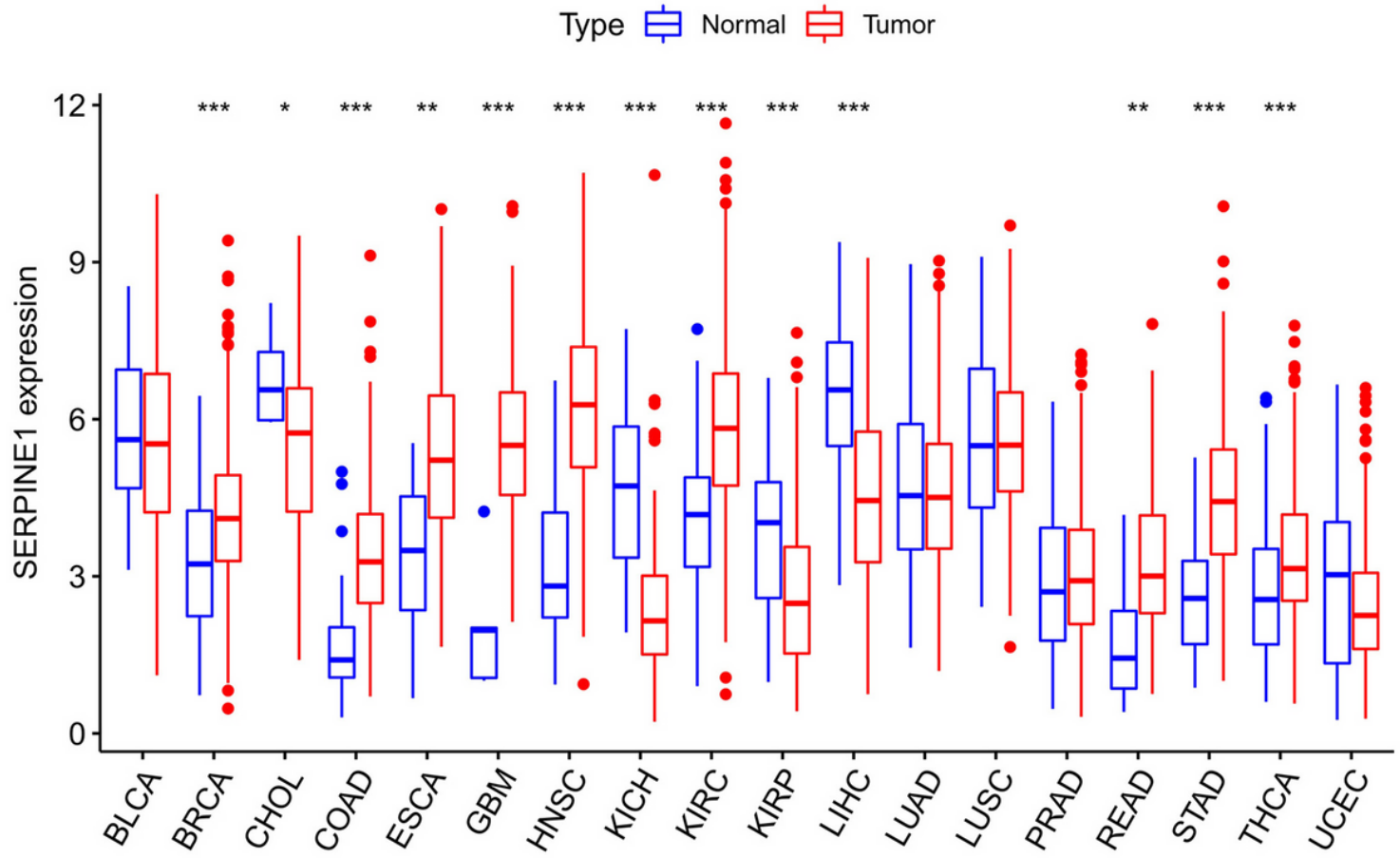
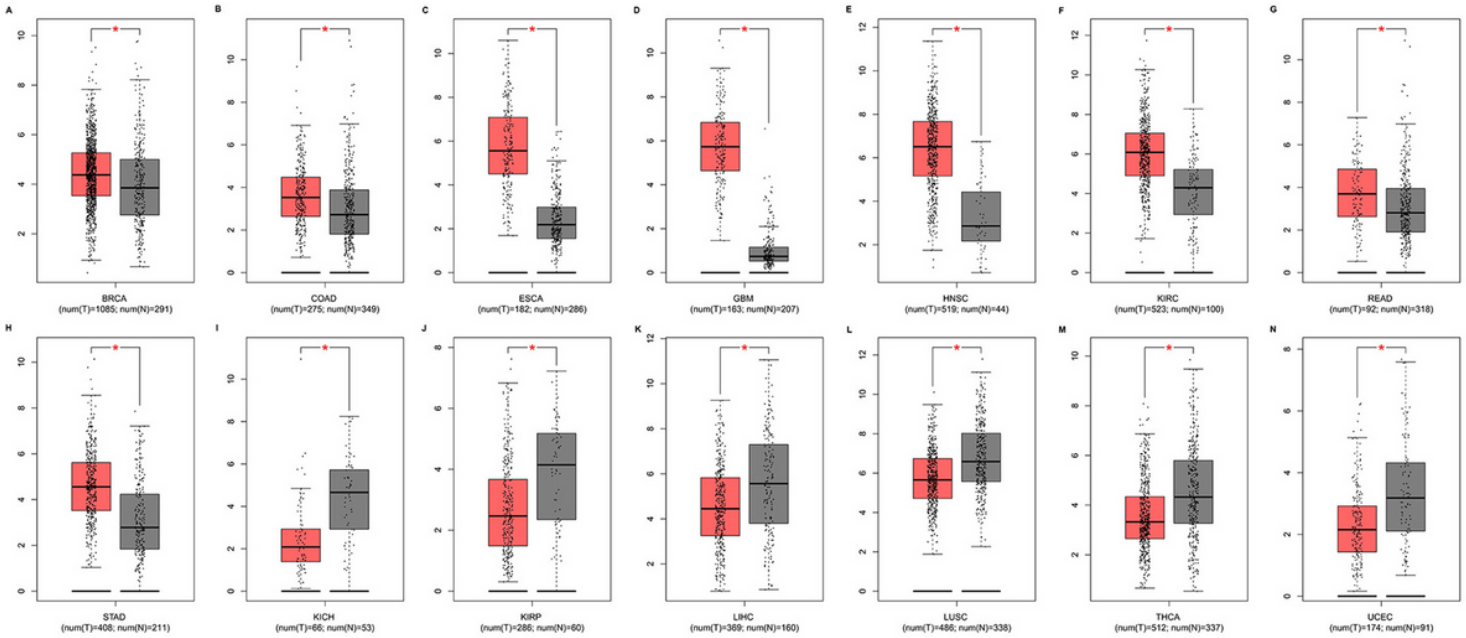


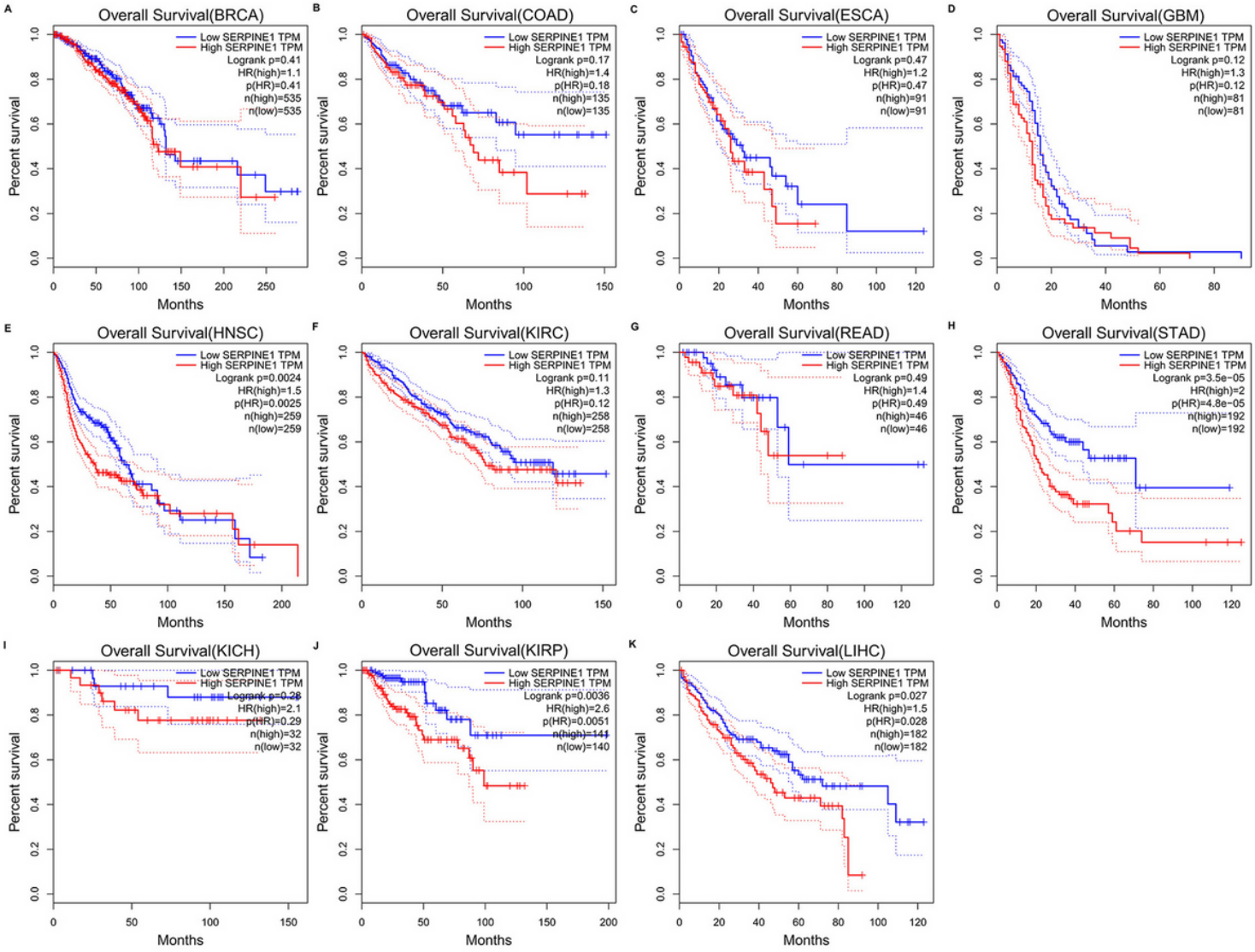

**Figure 1**

The expression of SERPINE1 in 18 types of human cancer based on TCGA cancer and normal data. *p value < 0.05; **p value < 0.01; ***p value < 0.001.



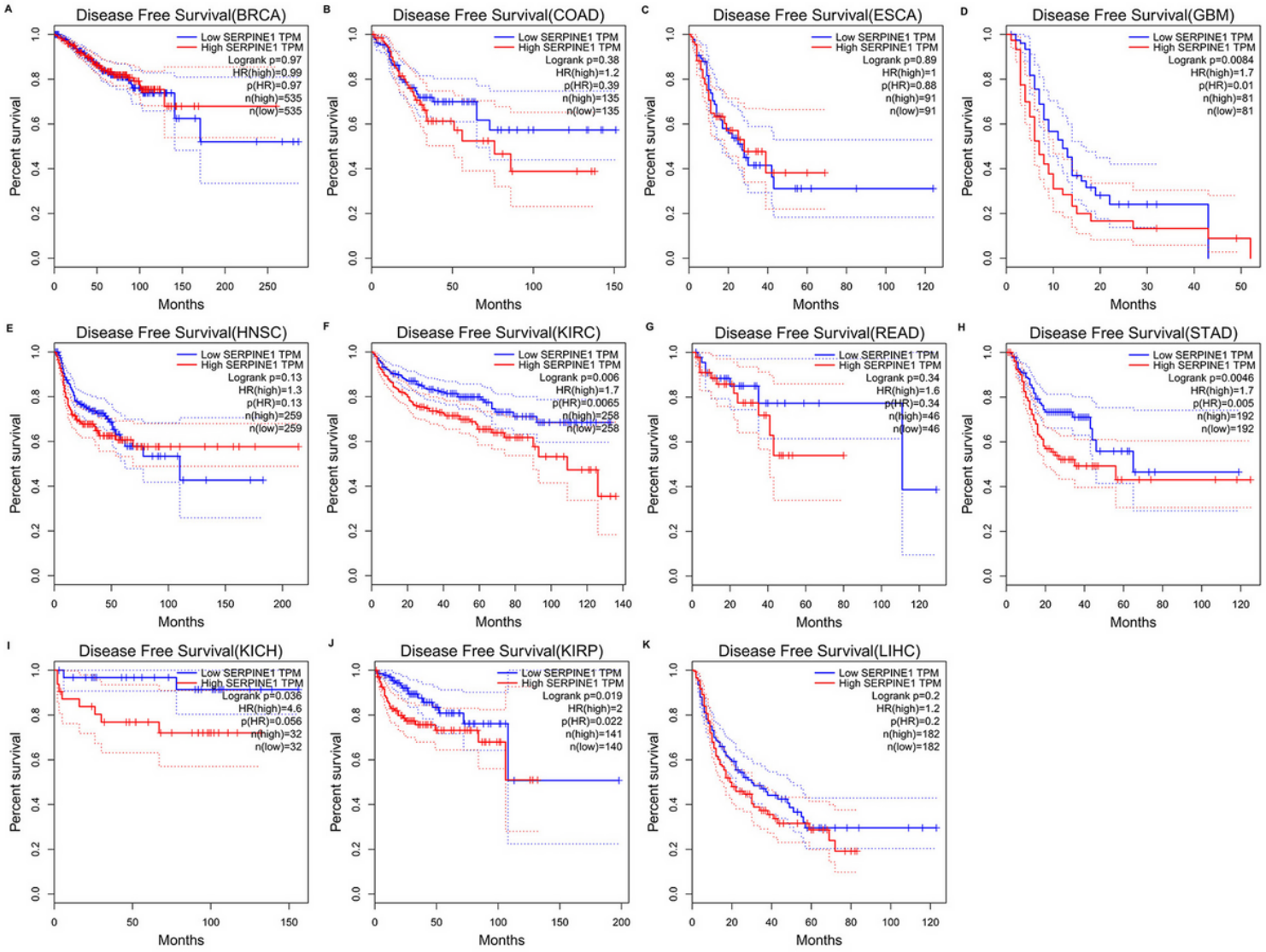
## Figure 2

(**A–N**) SERPINE1 expression in TCGA BRCA **(A)**, COAD **(B)**, ESCA**(C)**, GBM **(D)**, HNSC **(E)**, KIRC **(F)**, READ **(G)**, STAD **(H)**, KICH**(I)**, KIRP **(J)**, LIHC **(K)**, LUSC**(L)**, THCA**(M)** and UCEC **(N)** tissues compared with corresponding TCGA and GTEx normal tissues. *p value < 0.05.



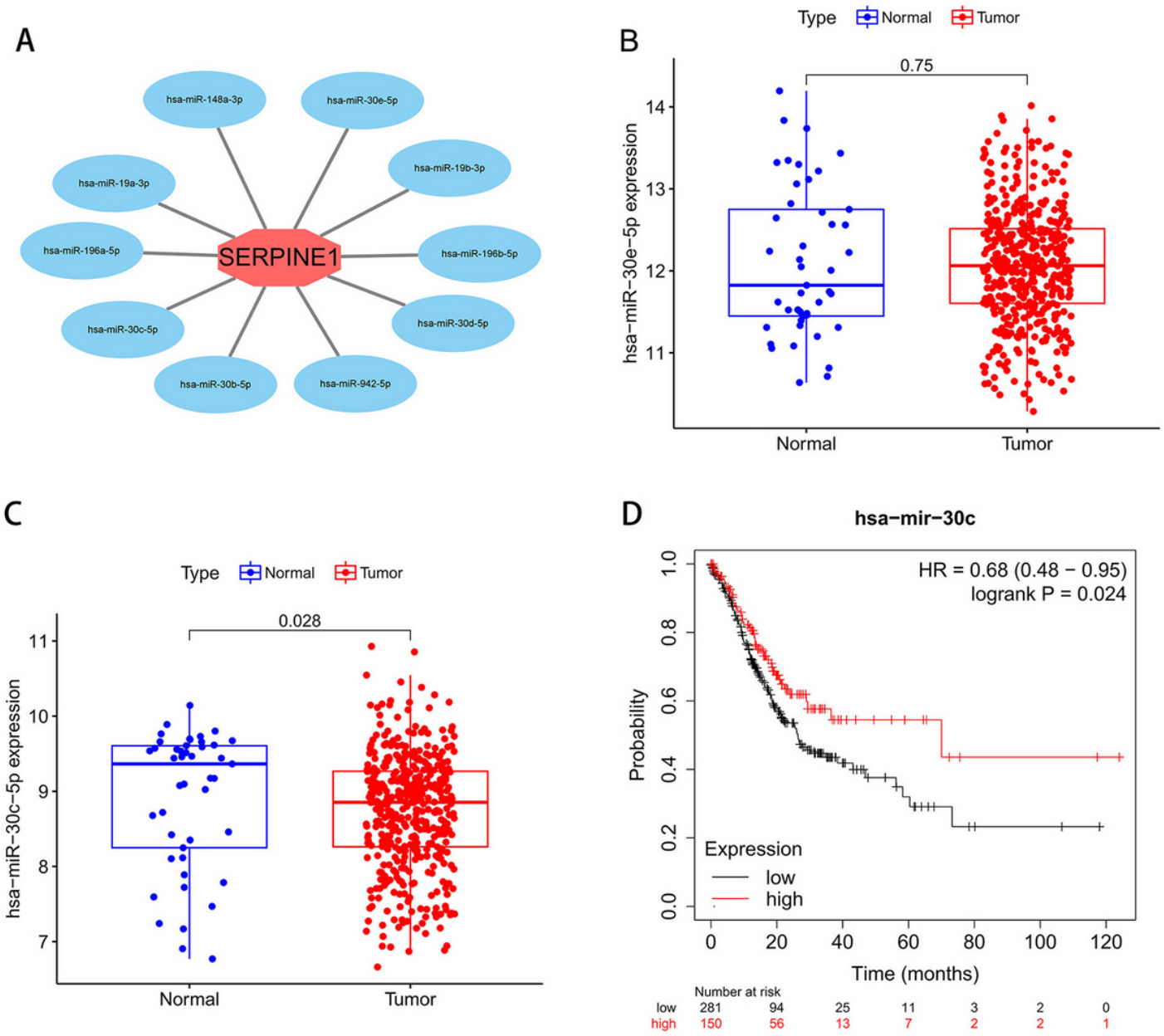

## Figure 3

The overall survival (OS) analysis for SERPINE1 in various human cancers identified by GEPIA database. **(A–K)** The OS plot of SERPINE1F in BRCA **(A)**, COAD **(B)**, ESCA**(C)**, GBM **(D)**, HNSC **(E)**, KIRC **(F)**, READ **(G)**, STAD **(H)**, KICH**(I)**, KIRP **(J)**, LIHC **(K)**.



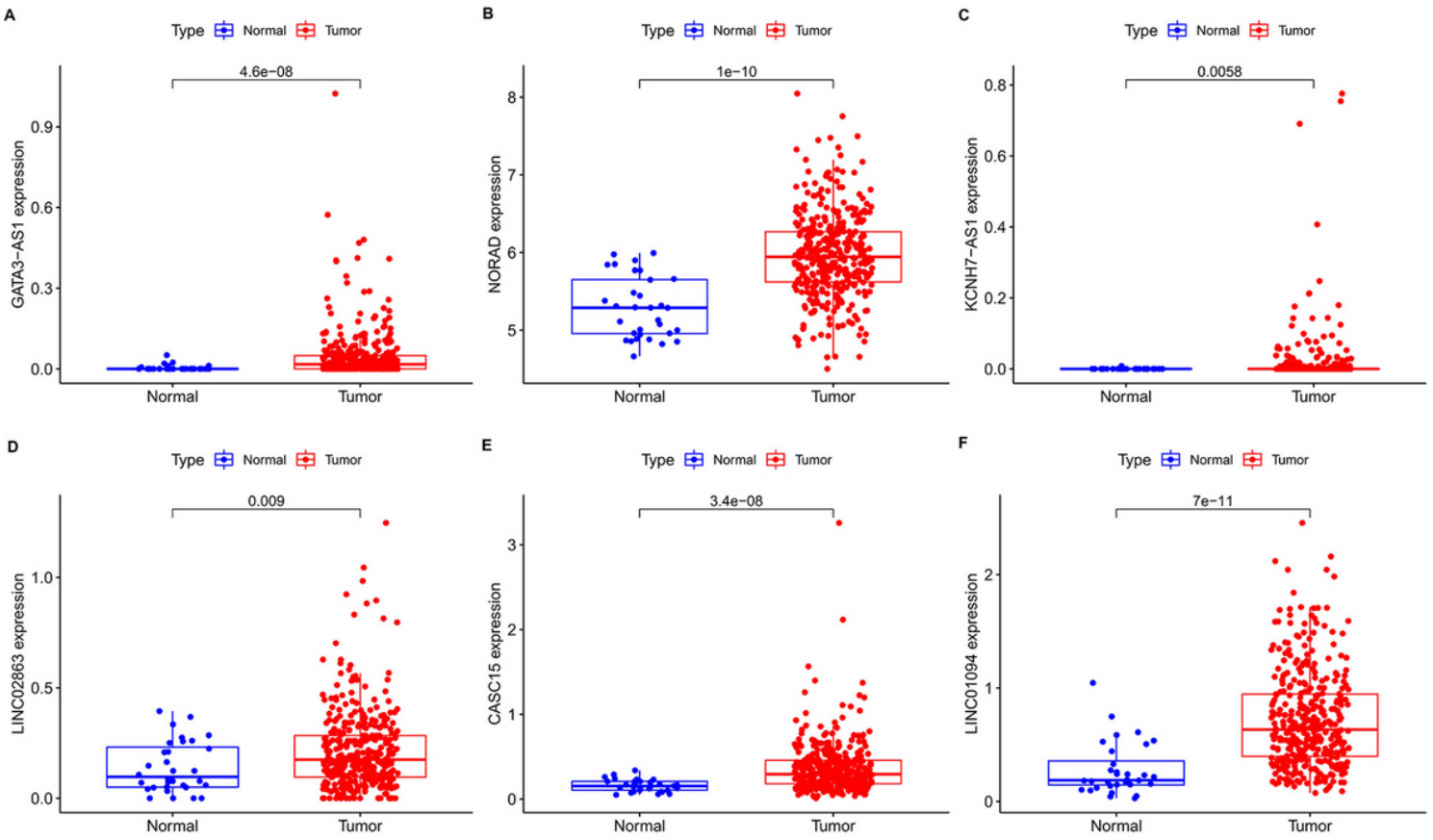

## Figure 4

The disease-free survival (RFS) analysis for SERPINE1 in various human cancers identified by GEPIA database. **(A–K)** The OS plot of SERPINE1F in BRCA **(A)**, COAD **(B)**, ESCA**(C)**, GBM **(D)**, HNSC **(E)**, KIRC **(F)**, READ **(G)**, STAD **(H)**, KICH**(I)**, KIRP **(J)**, LIHC **(K)**.




## Figure 5

identified and analysis on upstream miR-30c-5p of SERPINE1. **(A)** The miRNA-SERPINE1 regulatory network established by cytoscape software. **(B)** The expression of miR-30e-5p in STAD and control normal samples. **(C)** The expression of miR-30c-5p in STAD and control normal samples. **(D)** The prognostic value of miR-30c-5p in STAD assessed by Kaplan-Meier plotter.

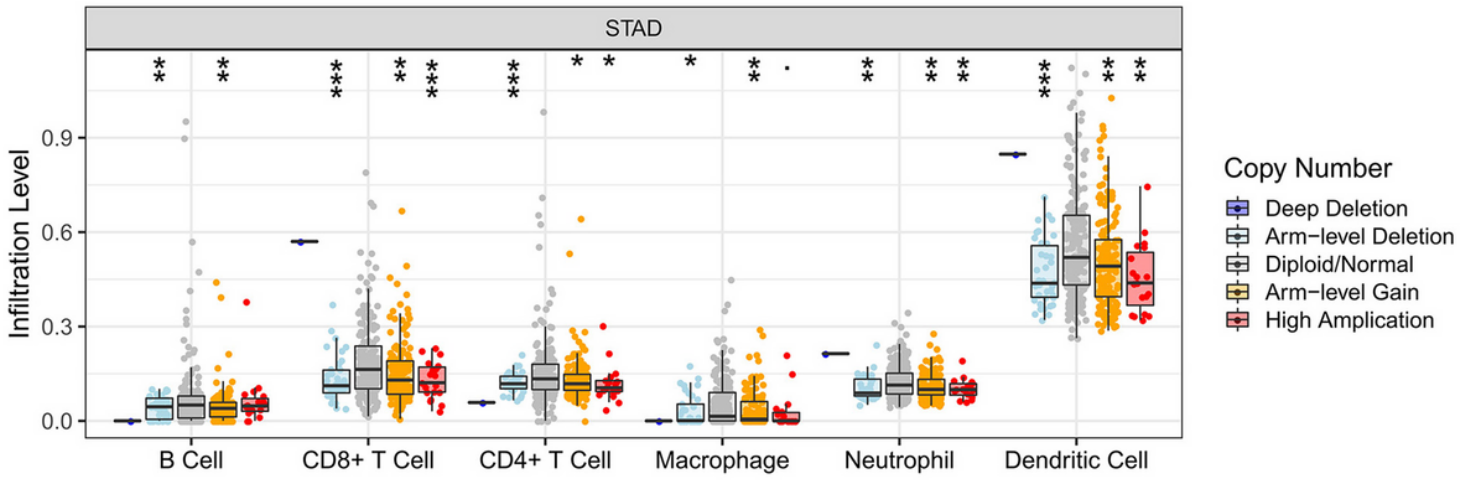



## Figure 6

**(A-F)** The expression level of 6 upstream lncRNAs of miR-30c-5p in STAD and control normal samples.

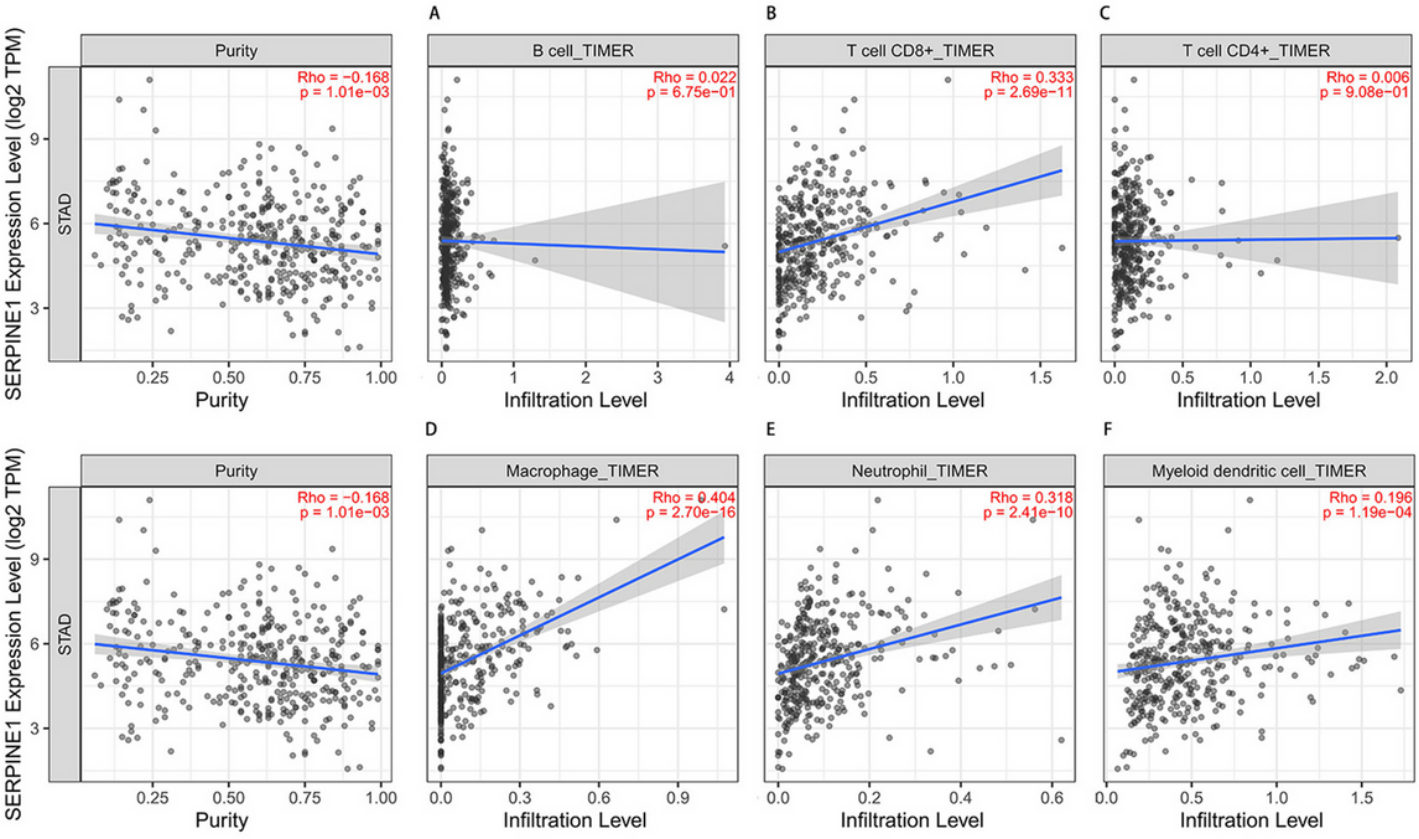
## Figure 7

Survival analysis for upstream lncRNAs of miR-30c-5p in STAD. **(A-C)** The prognostic value of LINC02863**(A)**, CASC15**(B)**, LINC01094**(C)** in STAD assessed by Kaplan-Meier plotter. **(D-E)** The OS and RFS analysis for CASC15**(D)** and LINC01094**(E)** in STAD by using GEPIA. *p value < 0.05.



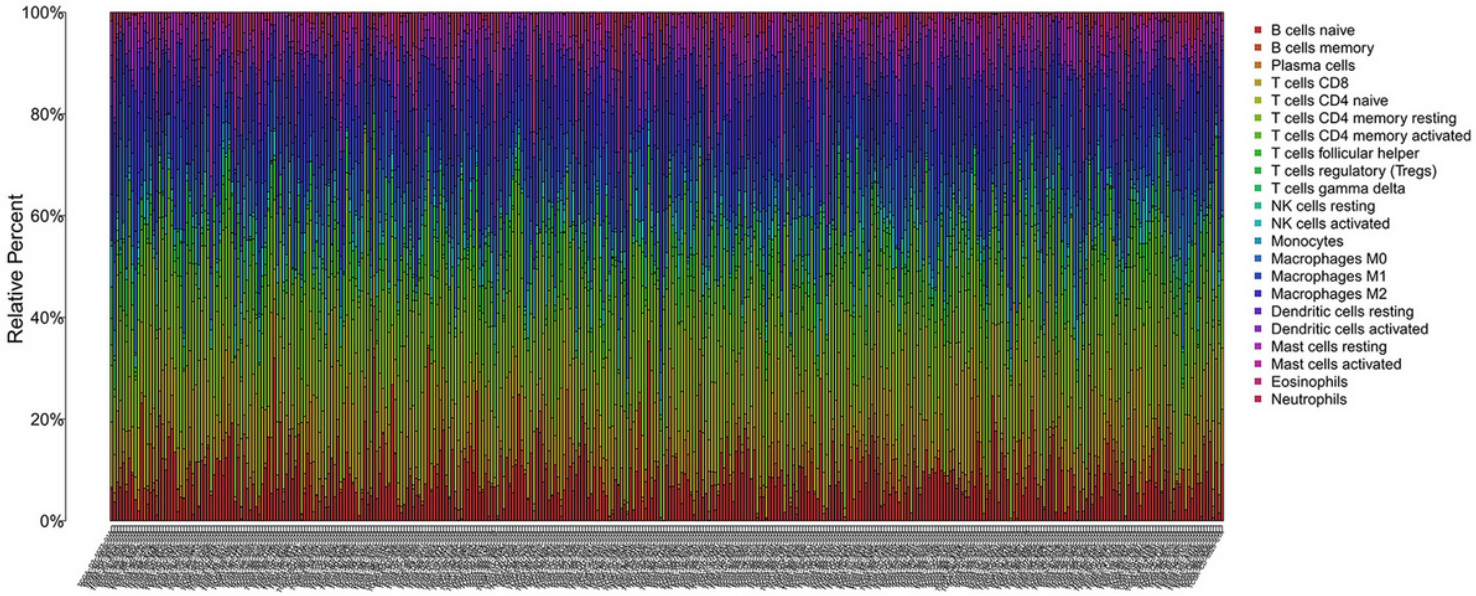

## Figure 8

The infiltration level of various immune cells under different copy numbers of SERPINE1 in STAD.



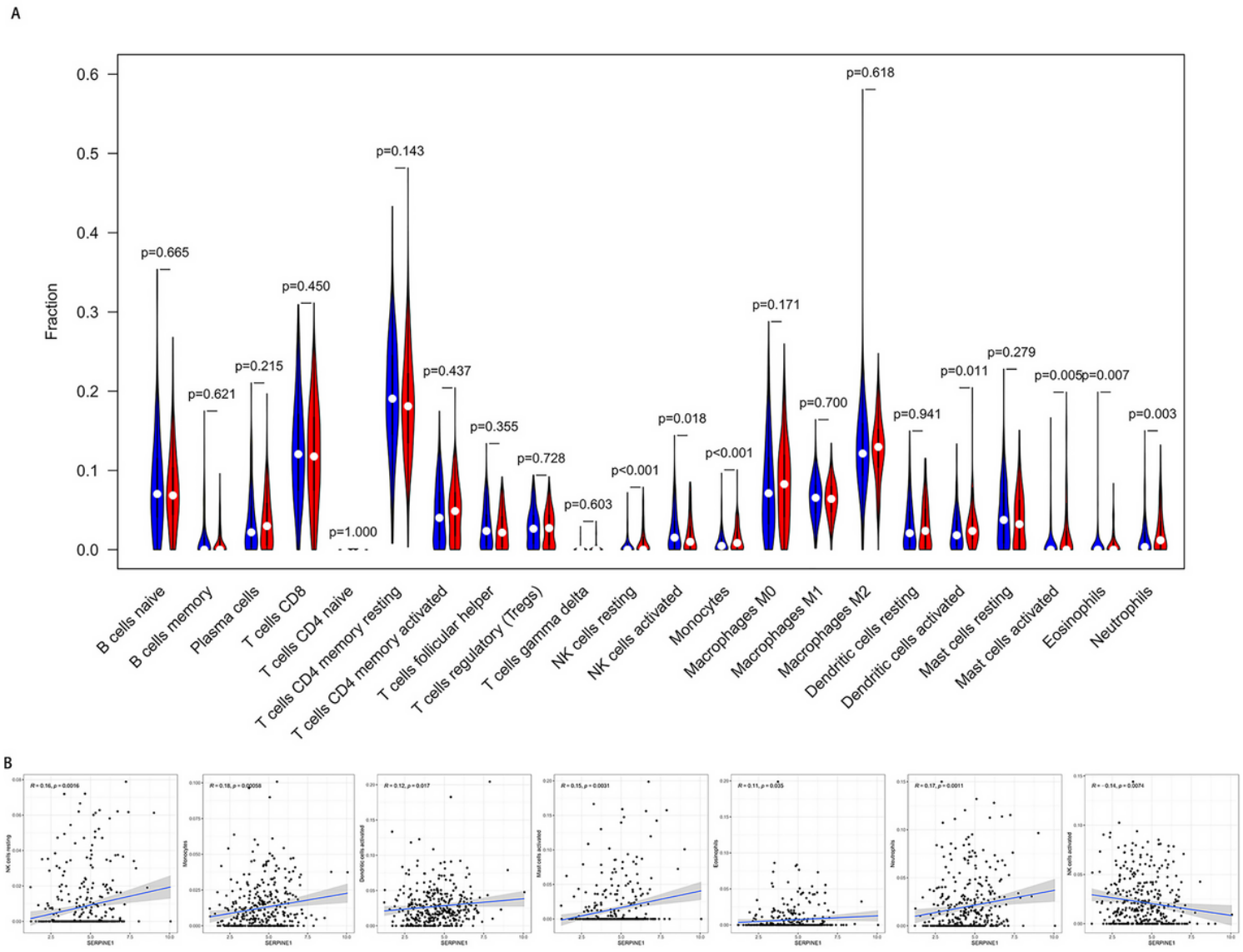

## Figure 9

The correlation of SERPINE1 expression level with B cell **(A)**, CD8+T cell **(B)**, CD4+T cell **(C)**, Macrophage **(D)**, Neutrophil **(E)**, or Dendritic cell **(F)** infiltration level in STAD. p value < 0.05.




## Figure 10

The relationship between the genes and immune cell infiltration. Bar plot showed the proportion of 22 immune cells with each sample.




## Figure 11

**(A)**Violin plot showed the expression difference of 22 immune cells between high expression group with low expression group in STAD tissues. **(B)** The correlation of SERPINE1 expression level with NK cells resting**,** Monocytes, Dendritic cells activated, Mast cells activated, Eosinophils, Neutrophils or NK cells activated infiltration level in STAD.

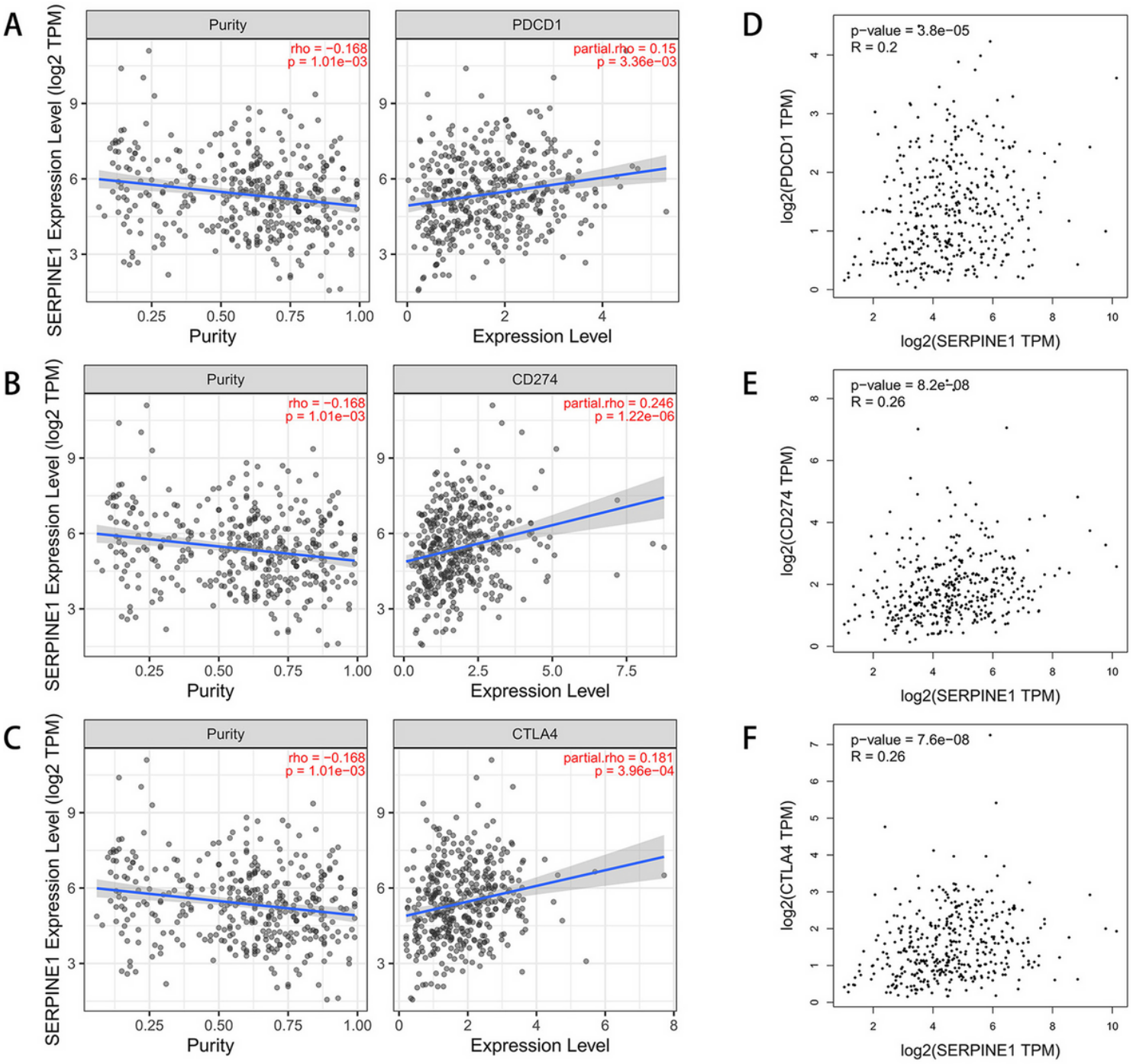



**Figure 12**

**(A-C)** Correlation of SERPINE1 with expression of PD-1, CD274 or CTLA-4 in STAD adjusted by purity using TIMER. **(D-F)** The expression correlation of SERPINE1 with PD-1, CD274 or CTLA-4 in STAD determined by GEPIA database.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx